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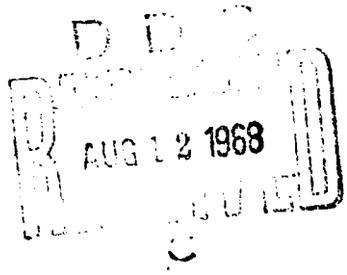

SOME NOTES ON THE NOURISHMENT OF CULICIDAE LARVAE
(B Factors and Proteinic Substances)

[Following is the translation of an article by E. Roubaud and P. Grenier of the Pasteur Institute in the French-language publication Bulletin de la Societe de Pathologie Exotique (Bulletin of the Society of Exotic Pathology, Vol XXXV, Nos 6-8, 1942, pp 215-219.]

Experiments in growing Stegomyia (Aedes aegypti) larvae conducted under aseptic conditions by W. Trager (1935-1941) emphasized the favorable action of B complex vitamins on the growth of the larvae [See Note]. For his part, Et. Sergent (1939), working on some ordinary, non-aseptic cultures, observed that when Anopheles maculipennis atroparvus larvae are fed only wheat flour they develop poorly and are subject to a high mortality rate, if a supplementary supply of vitamin B is not added to the medium. Under the influence of an excess in vitamin B, the A. maculipennis and the Culex pipiens larvae are ensured a better and more rapid growth. The action of other vitamins (A, C, D, E) is probably much less favorable.

([Note:] The action of these factors was probably in traces; for example, for riboflavin, at a concentration of 0.00008 mg (per cc).)

For the purpose of ascertaining a type of food that will permit easy laboratory cultures for supporting various strains of Culicidae, we made comparisons on them of a certain number of food powders that are more or less rich in B complex vitamins and in proteins. These experiments gave rise to the notes that are reported below.

Among the various types of foods used we shall retain the following products or mixtures as having especially yielded the most demonstrative results:

I. Wheat flour, 81.5% extracted.

II. Wheat flour plus purified casein (Hoffman-Laroche, free from vitamins A and D).

III. Wheat flour plus purified casein plus autolysate of yeast.

IV. Wheat flour plus purified casein plus dry yeast.

We worked with the following Culicidae species: Theobaldia annulata Schr., Culex pipiens pipiens, Anopheles maculipennis var. atroparvus V. Th.

Larvae proceeding from one and the same laying were distributed immediately after hatching, in glass containers, all of the same capacity. The various foods, reduced to the state of fine powders, were spread on the surface of the liquid in amounts that were appreciably equal and sufficient to form a thin surface film. The cultures were kept with the thermostat set at 26° C.

On the average, they were fed every two days; but every time a slight cloudiness indicated the beginning of bacterial pullulation that was apt to distort the results, the liquid medium and the food were completely replaced. Thus we were able practically to eliminate the major disturbing influence that microbian actions produce in cultures made under non-aspectic conditions.

Every day, in each batch, the moltings and, eventually, the dead larvae were counted.

The graphs given below (Figure 1), in which the relative rapidity of growth is expressed by the percentage of moltings at each stage, as a function of the development time (days), bring to light the following results for Theobaldia annulata cultures.

The best culture yields, both from the point of view of rapidity of development and of the number of imagoes that reached maturity, were obtained with mixture II, flour plus casein. With this mixture the liquid remains clear, without excessive bacterial pullulation; the larvae are vigorous and active, the moltings are well-grouped time-wise, almost simultaneous. The average larval evolution lasted an average of 14 days.

On the other hand, the mediums to which substances very rich in B complex vitamins (4 and 5) were added displayed very rapidly an excessive bacterial pullulation. The water became cloudy, gave off a nauseating odor and could result in a more or less high mortality. The evolution time observed with mixtures III and IV was essentially the same as with the preceding batch.

The use of wheat flour only also allowed us to ensure the normal growth of the larvae, but with a considerable delay in proportion to the results obtained with mixture II. The second molt occurred only toward the eighth day; that is to say, almost at the same time as the third one in the cultures fed on the wheat-casein mixture. Molting number 3 appears only toward the 16th day, and the nymph stage only toward the 26th day. Let us note that the individuals obtained were smaller than in the preceding cultures and the proportion of deaths was higher.

Experiments performed with Culex pipiens, under the same conditions, gave essentially the same results. On the other hand, with A. maculipennis a sizeable mortality, which occurs commencing with the first stages, prevents the conduct of experiments that must be taken up from that point of view under other conditions.

The result of these experiments is that, for Theobaldia and Culex larvae, the supply of vitamin B in wheat flour is in itself sufficient to ensure growth. It is not necessary to resort to a supplement of B complex substances in order to obtain a rapid growth of the larvae. A satisfactory supply of proteinic elements ensures this result, as is demonstrated by the cultures produced with mixture II, flour plus purified casein. In mixtures III and IV, enrichment with proteinic substances certainly played a larger part in accelerating growth than enrichment with B complex vitamins.

The slackening in growth observed when wheat flour was fed exclusively seems to be related primarily to an insufficiency of food of a proteinic nature.

This concept was subsequently verified. By substituting for the wheat flour a flour much more rich in proteinic substances, like soya flour, excellent growths are obtained. This flour is an optimum food for growing Culex and Stegomyia larvae. A comparison of the respective components of these two flours makes the favorable action of proteinic substances stand out very clearly. Because of their abundance, they make soya flour a much richer food for the larvae than wheat flour:

Wheat flour, 60% Extracted (According to Mme. Randouin's Data) Per 100 G. of Edible Portion	Soya Flour (Data of M. H. Haydalc, J. Econ. Ent., Vol 30, 1937)
Glucides 75%	Glucides 30.3%
Proteins 11%	Proteins 44.3%
Lipids 1%	Lipids 5.7%
Vitamin B . . . 24 I.U.	Vitamins B and B_2

To summarize, the experiments performed with the Theobaldia and Culex larvae show plainly that, although the B complex vitamins are indispensable in traces for the normal growth of the larvae, it is not necessary in order to produce their complete development to add substances to the wheat flour that enrich it in this group of vitamins. The rapidity and the perfection of the growth are chiefly a function of the abundance of proteinic elements present in the food apart from the B vitamins.

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COMMENT

R. PONS. -- Since vitamin B has, among its most important functions, that of acting on the metabolism of carbohydrates, I ask Mr. Roubaud whether the mediums utilized to study the evolution of mosquito larvae contained carbohydrates. If those ternary compounds were missing, we believe that it would be interesting to reconsider the action of vitamin B in the presence of certain sugars.

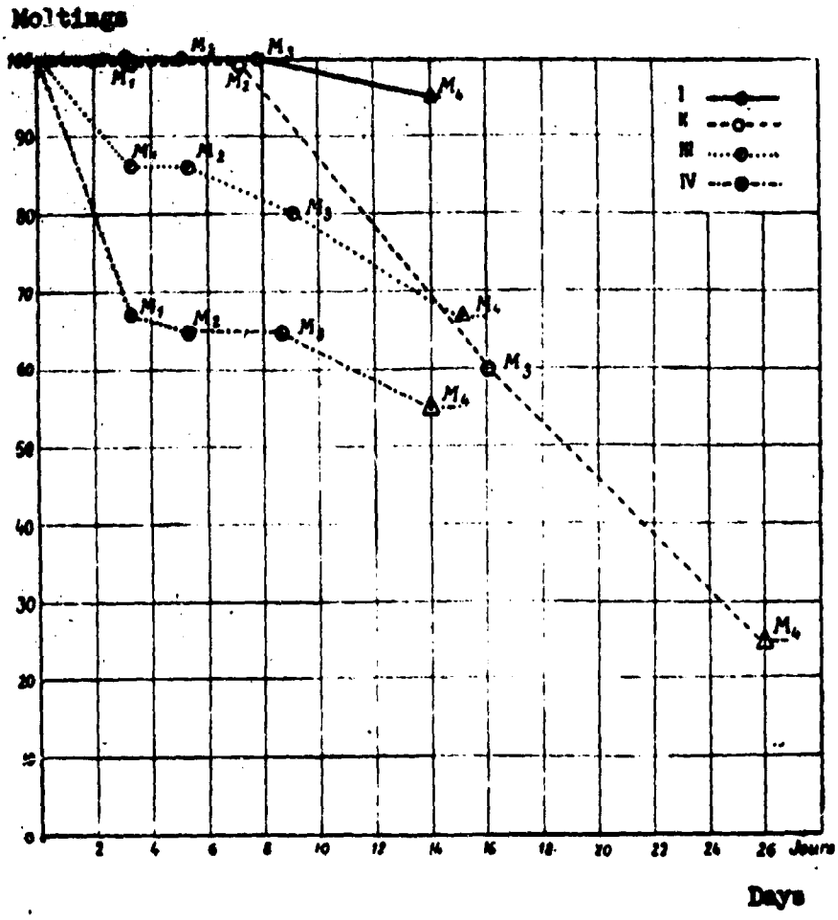

Figure 1